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Genetic Diversity of Insulin Resistance and Metabolic Syndrome

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Abstract

A key in the etiology of a cluster of metabolic syndrome such as hyperglycemia, dyslipidemia, and obesity is known for insulin resistance, which is becoming a major global public health problem. Extensive studies have revealed many genetic factors for both insulin resistance and the components of metabolic syndrome. Advanced modern genotyping methods including genome-wide association studies and next-generation sequencing have allowed for the identification of both common and rare genetic variants related to these chronic disease-associated traits. Multiple genotype–phenotype studies are also needed to identify new and accurate genetic biomarkers in these conditions. The purpose of this chapter is to present genetic variants related to the pathogenesis of metabolic syndrome and insulin resistance and is to review the relevance between insulin resistance and metabolic syndrome clusters in terms of genetic diversity.

Keywords: metabolic disorders, genetic variation, genetic biomarker, genetic analysis

1. Introduction

Metabolic syndrome (MetS), known as syndrome X, Deadly Quartet, or insulin resistance syndrome is characterized by a cluster of metabolic risk factors such as obesity, hypertension, dyslipidemia, and elevated fasting plasma glucose [1]. The metabolic risk factors can result in type 2 diabetes (T2D) and cardiovascular disease (CVD) that are due to both genetic and environmental factors [2, 3]. For these reasons, MetS is becoming a global epidemic. The prevalence of MetS is estimated at 11.9–37.1% in Asia-Pacific region [4], 11.6–26.3% in Europe [5], and 22–24% in North America [6].

One of the primary mediators of MetS is known for insulin resistance (IR), which is a pathological state of improper cellular response to the hormone insulin in insulin-dependent cells such as skeletal muscle and adipose tissue [7]. IR is present in the majority of people with many metabolic disorders such as MetS and T2D. IR plays a crucial role in the pathophysiology of both T2D and CVD [7] but inversely related to insulin sensitivity in insulin-dependent tissues [8]. Clinical risk factors such as obesity, dyslipidemia, inflammation, hyperinsulinemia, and dysglycemia are also known to affect IR.

Although environmental factors such as lifestyle and socioeconomic status contribute to the development of IR and MetS, both IR and MetS are also being determined by genetic factors, as strongly evidenced by early familial genetic studies [9–11]. Based on these studies, advanced genetic analysis technologies such as genome-wide association studies (GWAS) and next-generation sequencing (NGS) are extensively being used to identify both common and rare genetic variants related to these metabolic disorder-associated traits.

This chapter is to present an overview of genetic variants involved in the pathogenesis of MetS and IR and to review the relevance between IR and MetS clusters in terms of genetic diversity.

2. Heritability of MetS and IR

The pieces of evidence for the heritability and co-occurrence of the metabolic traits have been revealed through early familial and twin genetic studies. The heritability of MetS, as defined by NCEP:ATPIII (National Cholesterol Education Program Adult Treatment Panel III) criteria, was estimated to be 24% ($p = 0.006$) in the Northern Manhattan Family Study, which was conducted in 803 subjects from 89 Caribbean-Hispanic families [9]. Each component of MetS has also an important genetic basis. The heritability was estimated at 46% for waist circumference (WC), 24% for fasting glucose, 60% for HDL-cholesterol, 47% for triacylglycerol (TAG), and 16% for systolic and 21% for diastolic blood pressure (BP). In the Linosa Study including 293 Caucasian native subjects from 51 families (123 parents and 170 offsprings), the heritability of MetS, as defined by NCEP:ATPIII, was estimated to be 27% ($p = 0.0012$) [12]. Among its components, the heritability for blood glucose and high-density lipoprotein (HDL)-cholesterol was 10% and 54%, respectively. The highest heritability was observed in the clustering of central obesity, hypertriglycemia, and low HDL-cholesterolemia (31%, $p < 0.001$). In an early study including 2508 adult male twin pairs, the accordance for the clustering of hypertension, diabetes, and obesity in the same individuals was 31.6% in monozygotic pairs and 6.3% in dizygotic pairs [13]. These early pieces of evidence have spurred many studies to find genetic determinants of MetS.

Although common genetic variants related to IR have been identified, these variants are known to make up only 25–44% of the heritability of IR [14–16]. For this reason, it is necessary to find low-frequency and rare genetic variations that affect the heritability of MetS and IR.

3. Genetic variants of MetS and IR

Significant progress has been made over the past decade to identify the genetic risk factors associated with the various traits of MetS. Although the complexity of MetS makes the identification of a genetic component of the disorder difficult, pieces of evidence for genetic determinants of MetS have been revealed through the linkage analysis approach, candidate gene association studies, GWAS, epigenetic studies, microRNAs, long-non-coding RNAs, system biological studies, and more recently NGS and whole-exome sequencing.

3.1 Linkage analysis approach

Many chromosomes and locus associated with MetS or its components or a combination of some of its components have been identified through linkage

analysis. This approach has identified candidate quantitative trait loci (QTL). In 2209 subjects from 507 Caucasian families, a QTL associated with body mass index (BMI), WC, and fasting plasma insulin on chromosome 3q27 was identified, which includes genes such as the solute carrier family 2 of the facilitated glucose transporter (*GLUT2*) [17].

In a study including 456 Caucasian (white) and 217 African-American (black) subjects from 204 families, evidence of linkage for increased body fat, abdominal visceral fat, TAG, fasting glucose, fasting plasma insulin, blood pressure, and decreased HDL-cholesterol was identified on chromosome 10p11.2 and 19q13.4 and 10q13.4 in white [18]. In black subjects, the linkage was identified on chromosome 1p34.1 [18].

In a study including four ethnic groups (Caucasian, Mexican-American, African-American, and Japanese-American), evidence of linkage of MetS traits (weight/waist, lipid factor, and BP) was identified, where there is a strong linkage on chromosome 2q12.1-2q12 for Caucasian subjects and 3q26.1-3q29 for Mexican-American subjects [19].

Genetic data were obtained for 2467 subjects from 387 three-generation families and 1082 subjects from 256 sibships, where a genomic region on chromosome 2 included a pleiotropic locus contributing to the clustering of multiple metabolic syndrome (MMS)-related phenotypes (BMI, waist-to-hip ratio (WHR), subscapular skinfold, TAG, HDL-cholesterol, homeostasis model assessment (HOMA) index, plasminogen activator inhibitor-1-antigen, and serum uric acid) [20].

In a study including 250 German families, a genome-wide linkage scan for T2D supports the existence of MetS locus on chromosome 1p36.13 and T2D locus on chromosome 16p12.2 [21].

In a study with 715 individuals in 39 low-income Mexican American families, strong evidence of a major locus near markers *D1S1597* and *D1S407* on chromosome 1p36.21 that influences variation in symptomatic or clinical gallbladder disease through a genome-scan and linkage approach was revealed [22].

3.2 Candidate gene association studies

Candidate gene association studies identify and investigate many candidate genes that regulate biological processes related to MetS. Analysis of the mutation burden of candidate genes is among the first methods used to uncover MetS genes. Especially, the association of MetS and single nucleotide polymorphisms (SNPs) in related genes has been examined in many studies.

An association with MetS for 8 SNPs that are mostly in 25 genes involved in lipid metabolism was revealed in 88 studies with 4000 subjects. In these studies, the minor allele of C56G (*APOA5*), T1131C (*APOA5*), rs9939609 (*FTO*), C455T (*APOC3*), rs7903146 (*TCF7L2*), C482T (*APOC3*), and 174G > C (*IL6*) were more prevalent in subjects with MetS but the minor allele of Taq-1B (*CETP*) was less prevalent in those [23].

The association of *HSD11B1* variants and *HSD11B1* expression in abdominal adipose tissue with T2D, MetS, and obesity was identified in 802 studies. Especially, a polymorphic variant was identified to be related to T2D in a study including Pima Indians, and an association between MetS with another polymorphic variant at the *HSD11B1* gene was identified in an Indian study. However, most studies did not find an association between *HSD11B1* polymorphic variants and T2D, MetS, and obesity, suggesting that the variants may play a minor role to develop obesity, T2D, and MetS [24].

A meta-analysis study including 25 reports revealed an association of *ADIPOQ* rs2241766 and rs266729 polymorphisms with MetS in the Chinese population,

where the G allele of rs2241766 increased the risk of MetS but no relevance to rs266729 was found [25].

In a study including 442 adults with MetS, it was revealed that *APOE* genotype affected IR, apolipoprotein (apo) CII, and CIII depending on plasma fatty acid (FA) levels in MetS. Elevated n-3 polyunsaturated FA (PUFA) was related to lower concentration of apo CIII in *E2* carriers and elevated C16:0 was related to IR in *E4* carriers. Decreased long-chain n-3 PUFA was associated with reduced apo CII level in *E2* carriers, after FA intervention. These results suggest that subjects with MetS may benefit from personalized dietary interventions based on *APOE* genotype [26].

A meta- and gene-based analysis including 18 studies was carried out to investigate the association of fat mass and obesity-related *FTO* gene polymorphisms with MetS, suggesting that *FTO* is strongly related to MetS ($p < 10^{-5}$) [27].

BALB/c mice are known to be resistant to a high-fat diet (HFD)-induced obesity. A recent study demonstrated that *Nod2*^{-/-} BALB/c mice developed HFD-dependent obesity and risk factors of MetS such as hyperglycemia and hyperlipidemia. Interestingly, *Nod2*^{-/-} HFD mice showed changes in the composition of gut flora and also delivered sensitivity to hyperglycemia, steatosis, and weight gain to wild type germ-free mice. Therefore, these results suggest that not only *Nod2* plays a novel role in obesity but also that *Nod2* and *Nod2*-regulated gut flora protect BALB/c mice from diet-induced obesity and metabolic disorders [28].

More recently, a multiple-genotype and multiple-phenotype analysis of a gene-based SNP set has been performed to identify new susceptible variants associated with MetS in 10,049 Korean individuals [29]. In this study, 27 SNP pairs were associated with MetS in the discovery stage and also replicated. Of these SNPs, 3 SNP pairs in each *SIRT2*, *UBASH3B*, and *CUX2* gene were significant in the multiple-SNP and multiple-phenotype analysis rather than in the single-SNP and multiple-phenotype analysis. Especially, an association of MetS with an intronic SNP pair, rs7107152 ($p = 3.89 \times 10^{-14}$) and rs1242229 ($p = 3.64 \times 10^{-13}$), in *SIRT2* gene at 11q23.3 was found. These 2 SNPs are also associated with the expression of *SIRT2* and *TAGLN* that promote insulin secretion and lipid metabolism, respectively. These results suggest the usefulness of the multiple-genotype and multiple-phenotype analysis platform to identify new genetic loci in complex metabolic disorders such as MetS.

Although candidate genetic association studies have reported many genetic variations associated with MetS, often these results have not been replicated in other populations and been identified through GWAS. These examples include polymorphisms in or near genes encoding *GAD2*, *ENPP1*, and *SCL6A14*. Moreover, most of the identified genes underlie only one MetS trait. Few exceptions contain mutations in *ADIPOQ* related to hypertension, T2D, and dyslipidemia. Other examples contain mutations in *FOXC2*, *SREBP1*, *NR3C1*, and *GNB3* genes.

3.3 GWAS

GWA studies are an approach used to analyze an association of SNPs in subjects with MetS or IR and to date, being carried out by many researchers.

3.3.1 Genetic diversity of MetS

Over the past 10 years, GWAS have identified many genetic variants associated with each trait of MetS. Many genetic loci associated with lipid levels were discovered and refined by GWAS which identified 157 loci related to lipid levels at $p < 5 \times 10^{-8}$, including 62 loci not previously related to lipid levels [30]. Among the loci, 39 loci were associated with TAG levels and 71 with HDL-cholesterol.

Several loci associated with each component of MetS have pleiotropic effects on two or more traits related to MetS.

A GWA meta-analysis including 76,150 subjects showed that the rs2943634 variant near *IRS1* was associated with an elevated visceral to subcutaneous fat ratio, IR, dyslipidemia (higher TAG and lower HDL-cholesterol), risk of T2D, and reduced adiponectin levels [31]. Genetic variants in the *GCKR* gene were linked to fasting glucose levels [32], TAG [33], and non-alcoholic fatty liver disease [34]. Variants for obesity in/near *FTO* and *MC4R* genes were associated with specific measures of adiposity such as WC [35], HDL-cholesterol levels [30], IR [36, 37], and risk of T2D [35]. Variants in the *GRB14* gene were also linked to BMI-adjusted WHR [38], T2D [39], and fasting insulin levels.

In a GWAS comparing T2D subjects ($n = 1924$) and control ($n = 2938$) for autosomal SNPs ($n = 490,032$), SNPs in *FTO* gene region on chromosome 16 were identified to be strongly associated with T2D (e.g., rs9939609, $OR = 1.27$, $p = 5 \times 10^{-8}$). This strong association was furthermore reproduced by analyzing SNP rs9939609 in T2D subjects ($n = 3757$) and controls ($n = 5346$) ($OR = 1.15$, $p = 9 \times 10^{-6}$) [35]. However, some of these variants were also associated with MetS, suggesting that genes such as *FTO*, *MC4R*, and *IRS1* play important roles in the progression of MetS [40]. Especially, among several obesity-related loci found to be related to MetS-related traits in the GWAS studies, *FTO* and *MC4R* genes are considered to be the strongest candidates for body weight control, and *IRS1* is known to have an important effect on IR. These results may provide valuable information to understand the role of genetic control of adiposity and IR in the development of MetS.

GWA studies of MetS as a whole or a combination of its traits have also identified a number of both common and rare genetic variants. A GWA study was conducted to identify common genetic variants of MetS and its related components in 4560 Indian Asian men with a high prevalence of these conditions. In this study, no genetic variation showed an association with MetS as a whole. However, several variations were related to single components. Especially, 2 SNPs near *CETP*, 2 at 8p21.3 near *LPL*, 2 at 11q12.2 near *FADS1* and *FADS2*, and 1 at 21q22.3 near *FLJ41733* associated with HDL-cholesterol ($p < 10^{-6}$), and 1 SNP near *TCF7L2* associated with T2D ($p < 10^{-6}$) were identified [41].

A study by the STAMPEDE Consortium included 13 independent studies, comprising a total of 22,161 subjects of European ancestry, was conducted to find genetic determinants contributing to the correlated architecture of MetS traits, using MetS as a whole or pairs of its components as phenotypes [42]. In this study, the 5 SNPs in *LPL*, *APOA5* cluster (*ZNF259*, *BUD13*, and *APOA5*), and *CETP* genes were found to be associated with MetS. Especially, a total of 27 genetic variants in or near 16 genes were associated with bivariate combinations of 5 MetS traits, including variants in *LIPC* (chromosome 15q21-q23) associated with HDL-cholesterol-fasting glucose (rs2043085, $p = 1.3 \times 10^{-8}$) and with WC-HDL-cholesterol (rs10468017, $p = 5.5 \times 10^{-8}$), *ABCB11* (chromosome 2q24) associated with HDL-cholesterol-fasting glucose (rs569805, $p = 8.5 \times 10^{-8}$) and with HDL-cholesterol-TAG (rs2954026, $p = 7.9 \times 10^{-9}$), *TRIB1* (chromosome 8q24.13) associated with TAG-BP (rs2954033, $p = 8.5 \times 10^{-9}$), *TFAP2B* (chromosome 6p12) associated with WC-fasting glucose (rs2206277, $p = 1.3 \times 10^{-7}$), *LOC100128354* (chromosome 11q21) and *MTNR1B* associated with BP-fasting glucose (rs1387153, $p = 8.1 \times 10^{-9}$), HDL-cholesterol-fasting glucose (rs1387153, $p = 2.4 \times 10^{-9}$), and TAG-fasting glucose (rs10830956, $p = 4.8 \times 10^{-11}$), *LOC100129500* (chromosome 19q13.2) associated with HDL-cholesterol-TAG (rs439401, $p = 1.0 \times 10^{-8}$), and *LOC100129150* variants with HDL-cholesterol-TAG (rs9987289, $p = 1.1 \times 10^{-8}$) and HDL-cholesterol-WC (rs9987289, $p = 3.7 \times 10^{-8}$) [42]. These common genetic variations can partly explain the covariation in the MetS traits.

In a study for susceptibility loci associated with MetS and its traits was conducted in four Finnish cohorts consisting of 2637 MetS cases and 7927 controls. One genetic variant (rs964184) in A *APOA1/C3/A4/A5* gene cluster region on chromosome 11, known as lipid locus was found to be associated with MetS in all 4 study samples ($p = 7.23 \times 10^{-9}$ in meta-analysis) and significantly associated with several very low-density lipoprotein (VLDL), TAG, and HDL metabolites ($p = 0.024\text{--}1.88 \times 10^{-5}$). Several genetic variants in or near 4 known loci related to lipids (*LPL*, *CEPT*, *APOA1/C3/A4/A5*, and *APOB*) were strongly associated with TAG/HDL/WC factors [43], but none was associated with 2 or more uncorrelated MetS traits. A polygenetic risk score (PRS), which was calculated as the number of alleles in loci associated with individual MetS traits, was significantly associated with MetS traits. These results suggest that genes associated with lipid metabolism pathways have crucial roles in the development of MetS. However, in this study, little evidence for pleiotropy associating obesity and dyslipidemia with the other MetS traits (hyperglycemia and hypertension) was found.

Genetic loci associated with the clustering of 6 MetS-related phenotypes (atherogenic dyslipidemia, vascular dysfunction, vascular inflammation, pro-thrombotic state, central obesity, and elevated plasma glucose) including 19 quantitative traits were identified by GWAS in 19,486 European American and 6287 African American Candidate Gene Association Resource Consortium participants [44]. In this study, 606 significant SNPs in and near 19 loci ($p = 2.13 \times 10^{-7}$) were identified in European Americans. Many of these loci were associated with at least one MetS-related trait domain and consistent with results in African Americans. Three new pleiotropic loci in or near *APOC1*, *BRAP*, and *PLCG1*, which were associated with multiple phenotype domains were identified. Several loci previously identified by GWAS for each trait of MetS, including *LPL*, *ABCA1*, and *GCKR*, were also associated with at least 2 trait domains. These results support the presence of genetic variants with pleiotropic effects on adiposity, inflammation, glucose regulation, dyslipidemia, vascular dysfunction, and thrombosis. Such loci could apply to uncover metabolic dysregulation and identify targets for early intervention.

3.3.2 Genetic diversity of IR

To date, many of the loci related to risks of developing IR have been identified and found to be associated with measures such as insulin sensitivity and secretion.

In an early meta-analysis, genetic variants related to IR were identified in 21 cohorts consisting of a non-diabetic group, which includes 46,186 subjects with measures of fasting glucose and 38,238 subjects with measures of fasting glucose and HOMA-IR. In additional 76,558 subjects, 25 SNPs were followed up with this approach, identifying 16 loci related to fasting glucose and 2 loci related to fasting insulin. In this study, several loci near *GCKR* including a new locus near *IGF1* were found to be associated with IR [32]. These results were replicated in a further 14 cohorts, which included 29,084 non-diabetic subjects with measures of fasting proinsulin, insulin secretion, and sensitivity [45]. Association of 37 risk loci for T2D with measures of insulin secretion, sensitivity, and processing and clearance was examined in 58,614 non-diabetic subjects and 17,327 subjects with measures of glycemic traits, revealing that the risk loci were grouped into 5 major categories including one cluster with 4 loci (*PPARG*, *KLF14*, *IRS1*, and *GCKR*) associated with IR [46].

A joint meta-analysis (JMA) approach has been developed to identify genetic variants associated with either fasting glucose and/or fasting insulin. This approach identified 6 loci that include 5 new variants associated with levels of fasting insulin (*IRS1*, *COBLL1-GRB14*, *PPP1R3B*, *PDGFC*, *UHRF1BP1*, and *LYPLAL1*) [47].

A large-scale meta-analysis including 133,010 subjects identified 17 loci significantly associated with fasting insulin. These loci included genes associated with other metabolic traits (*FTO*, *TCF7L2*, *PPARG*, *ARL15*, *RSPO3*, and *ANKRD55-MAP3K1*) and newly identified loci (*YSK4*, *FAM13A*, *TET2*, *PEPD*, and *HIP1*) [48]. In 2 further studies, these loci were used to make an IR PRS identify the relationship between variants associated with fasting insulin and the risk of each individual developing IR and T2D [49, 50]. The 2 studies identified that the IR GRSs were associated with decreased insulin sensitivity and lower BMI. In one of these 2 studies, a PRS was generated from 10 genetic loci that were related to lower HDL-cholesterol and higher TAG (*PPARG*, *IRS1*, *GRB14*, *PEPD*, *FAM13A1*, *PDGFC*, *LYPLAL1*, *RSPO3*, *ARL15*, and *ANKRD55-MAP3K1*). [49]. In the other study, 19 loci were used to generate their IR PGS and 11 risk variants were identified to be related to increased TAG and lower HDL-cholesterol along with a lower BMI [50]. In these studies, IR PRSs were used to highlight that IR can develop without obesity and high BMI.

IRS1 is a signaling adapter protein that is encoded by the *IRS1* gene in humans and a key factor of the insulin signaling pathway initiating the activation of phosphoinositide 3-kinase (PI3K) in response to insulin. The C allele at the SNP (rs2943641) near the *IRS1* gene was found to be associated with IR and hyperinsulinemia in a European population. Through functional studies, the risk allele was found to be associated with lower levels of basal *IRS1* protein and decreased PI3K activity during insulin infusion, indicating a causative role for the genetic variant on risk of IR [51]. The SNP (rs2943650) near *IRS1* was also associated with lower HDL-cholesterol, elevated TAG, IR, and lower body fat percentage [31]. Significant associations of the variants in *FTO* with fasting insulin and insulin sensitivity were identified [37]. The risk variant in or near *TCF7L2* was found to be associated with both impaired β -cell function and IR [52]. A variant in *NAT2* was also found to be associated with a measure of insulin sensitivity in four European cohorts of 2764 non-diabetic individuals [53], supporting a role for *NAT2* in insulin sensitivity. In this study, a variant of *NAT2* was found to be strongly associated with reduced insulin sensitivity that was independent of BMI. The A allele at the SNP (rs1208) was significantly associated with IR-related traits, including increased fasting glucose, total cholesterol and LDL-cholesterol, hemoglobin A1C (HbA1c), TAG, and coronary artery disease (CHD). IGF1 is functionally similar to insulin and controls growth and development. Lower levels of IGF1 were found to be associated with decreased insulin sensitivity [54], and the SNP (rs35767) in the *IGF1* gene suggested that the G allele has lower levels of IGF-1 compared to the A allele [55].

In a GWA study of a UK cohort of Indian-Asian and European ancestry, *MC4R* was found to be associated with both IR with measures of HOMA-IR and WC, and with higher frequencies of risk alleles found in the Indian-Asian cohort [36].

In a GWA study of a cohort with Indian ancestry, 2 loci near *TMEM163* were found to be associated with both reduced plasma insulin and HOMA-IR [56].

In a GWA study of an African-American cohort, the SNP (rs7077836) near *TCERG1L* and the SNP (rs17046216) in *SC4MOL* were found to be associated with both fasting insulin and HOMA-IR [57]. *ARL15* belongs to a family of intracellular vesicle trafficking, and its exact function remains unknown. However, variants in *ARL15* were found to be associated with decreased levels of adiponectin and risk of T2D, CHD, and IR as measured by HOMA-IR [58].

To date, approximately 60 loci related to the risk of IR have been identified through GWAS, and among them, the top 10 IR-related loci have been replicated in 2 GWA studies [48, 59]. They are in and near the noncoding regions of *IRS1* (rs2943645), *PPARG* (rs17036328), *GRB14* (rs10195252), *PEPD* (rs731839), *PDGFC* (rs6822892), *MAP3K1* (rs459193), *ARL15* (rs4865796), *FAM13A* (rs3822072),

RSPO3 (rs2745353) and *LYPLAL1* (rs4846565). The PRS including the risk alleles of the 10 loci was associated with the cardiometabolic phenotypes such as lower BMI, lower body fat percentage, smaller hip circumference, and decreased leg fat mass as well as the risk phenotypes such as higher fasting insulin and higher TAG levels. These results suggest that limited storage capacity of subcutaneous adipose tissue (SAT) and consequently the elevation of ectopic fat deposition may be associated with the genetic link with IR [48, 49].

3.4 Low-frequency and rare variants

Whole-genome and exome sequencing approaches as relatively new genetic analysis technologies are being used to pinpoint the effects of minor allele frequencies (MAF $\leq 5\%$) and rare variants (MAF $\leq 0.5\%$) on the heritability of metabolic disorders such as MetS and IR.

The genomes of 1092 individuals from 14 populations were analyzed by using both the whole-genome and exome sequencing methods to identify low-frequency and rare genetic variants across 14 populations in the 1000 Genome Project [60]. The reference panels gained from this project can capture up to 98% accessible SNPs at a frequency of 1% in related populations and also enable researchers to analyze common and low-frequency variants in each individual from various populations. The 38 million SNP panels from the 1000 Genomes Project gave near complete coverage of common and low-frequency genetic variation with MAF $\geq 0.5\%$ across European ancestry populations.

The European Network for Genetic and Genomic Epidemiology (ENGAGE) consortium carried out 22 GWAS to examine associations of genetic variants with WHR, fasting glucose, BMI, and fasting insulin in 87,048 individuals of European ancestry. This study identified two new loci for BMI, and fasting glucose and new lead SNPs at 29 loci including the SNP (rs1260326) near *GCKR* for fasting insulin [61].

Whole exome sequencing in a Danish cohort of 1000 individuals with T2D, BMI $>27.5 \text{ kg/m}^2$, and hypertension and of 1000 controls identified 70,182 SNPs with MAF $> 1\%$. Subsequent exome sequencing was performed in a two-stage follow-up in 15,989 Danes and a further 63,896 Europeans. This study showed associations of two common SNPs in *COBLL1* (MAF = 12.5%, OR = 0.88, $p = 1.2 \times 10^{-11}$) and *MACF1* (MAF = 23.4%, OR = 1.10, $p = 8.2 \times 10^{-10}$) with T2D and a low frequency variant in *CD300LG* (MAF = 3.5%, $p = 8.5 \times 10^{-14}$) with fasting HDL-cholesterol [62].

Although physiological functions of risk variants in *COBLL1* and *MACF1* remain still unclear, a risk variant rs72836561 at *CD300LG* was found to be associated with the decreased mRNA expression level of *CD300LG* in both skeletal muscle and adipose tissue, elevated intramyocellular lipid, and decreased insulin sensitivity, through a functional study. These results suggest an association between this variant and MetS traits [63].

Exome sequencing in an Icelandic population revealed that a low-frequency (1.47%) variant (rs76895963) in *CCND2* decreased the risk of T2D (OR = 0.53, $p = 5.0 \times 10^{-21}$) and was associated with elevated *CCND2* expression [64]. However, this variant was also associated with both greater height and higher BMI (1.17 cm per allele, $p = 5.5 \times 10^{-12}$ and 0.56 kg/m² per allele, $p = 6.5 \times 10^{-7}$, respectively).

In 2733 individuals from the Greenlandic population that were historically isolated, combination analyses of Cardio-MetaboChip based genotyping and exome sequencing revealed that a common variant in *TBC1D4* was associated with higher fasting glucose and decreased insulin sensitivity, resulting in decreased insulin-stimulated glucose uptake due to the variant [65].

Recently, whole-genome sequencing in a UK10K-cohort project consisting of 3781 healthy individuals with exome sequencing of 6000 individuals with either rare disease, severe obesity, or neurodevelopmental disorders has been performed to identify low-frequency and rare variants [66]. This project identified 24 million novel genetic variants including novel alleles associated with levels of TAG (*APOB*), adiponectin (*ADIPOQ*), and LDL-cholesterol (*LDLR* and *RGAG1*) from single-marker and rare variant aggregation tests and provided reference panels with increased coverage of low-frequency and rare variants. These panels are now being used to identify associations of low-frequency and rare variants with various traits related to health and disease.

3.5 Epigenetic determinants

Fatty acid-binding proteins (FABPs) play important roles in lipid metabolism and signaling. Dyslipidemia often occurs along with IR, obesity, and hypertension in individuals with MetS. The methylation status of CpG islands of a key regulator of lipid homeostasis, *FABP3*, is known as a quantitative trait associated with MetS phenotypes in humans. To identify if CpG methylation of *FABP3* affects MetS traits in 517 Northern European family populations, the CpG islands in the *FABP3* gene were profiled by a quantitative methylation analysis method. In this study, regional methylation was found to be strongly associated with plasma total cholesterol ($p = 0.00028$) and associated with LDL-cholesterol ($p = 0.00495$) [67]. Methylation at individual units was significantly associated with MetS traits such as insulin sensitivity and diastolic BP ($p < 0.0028$). These results suggest that DNA methylation of *FABP3* strongly affects MetS and might have important implications for insulin, lipids, and cardiovascular phenotypes of MetS.

Meanwhile, malnutrition in childhood, infancy, or fetus affects the prevalence of MetS in adults and their offspring [68], suggesting that maternal malnutrition affects gene expression in offspring through epigenetic mechanisms.

To date, most studies examining epigenetic changes related to MetS or IR have been conducted in animals and few studies have been conducted in humans. Therefore, further studies in humans are needed in the future.

4. CRISPR screen for genes affecting MetS or IR

Although many GWA studies are widely used to identify genetic loci associated with IR, it remains challenging to identify the causal gene in each locus [69]. Recently, structural and functional connections between GWAS loci and vicinal or distal genes were identified by chromosome conformation capture (3C) technology and expression quantitative trait loci (eQTL) studies [70, 71]. However, the 3C experiments are expensive and the eQTL studies cannot identify all the causal genes for a locus. Moreover, the 2 methods cannot pinpoint the causal genes and mechanisms related to the risk loci of IR. More recently, clustered regularly interspaced short palindromic repeats (CRISPR) knockout screening platform as an alternative method has been applied to pinpoint functions of new candidate causal genes at IR-associated loci in human preadipocytes and adipocytes [72]. This screening platform successfully characterized the functions of 10 new candidate causal genes at IR-associated loci. The 10 candidate genes (*PPARG*, *IRS-1*, *FST*, *PEPD*, *PDGFC*, *MAP3K1*, *GRB14*, *ARL15*, *ANKRD55*, and *RSPO3*) showed diverse phenotypes in the 3 insulin-sensitizing mechanisms, including lipid metabolism, adipogenesis, and insulin signaling, and the first 7 of these genes could affect all the 3 mechanisms. Additionally, 5 of 6 eQTL genes were identified as the top candidate causal genes (*IRS-1*, *GRB14*, *FST*, *PEPD*,

and *PDGFC*), and expression levels of these 5 genes in human subcutaneous adipose tissue were found to be associated with increased risk of IR. Interestingly, it was first revealed in this study that the *FST*, *PEPD*, and *PDGFC* are involved in the functions of adipose in IR. Despite these findings, little is known about other functions of these 3 genes in adipose tissue, which may include novel molecular mechanisms for cardio-metabolic disease. In this regard, studies will be needed to uncover new functions of these 3 genes in adipose tissue.

5. Conclusions

MetS and IR are central risk factors for the development of dyslipidemia, T2D, and CVD as well as complex metabolic traits. Many of the genetic variations implicated in the development of the MetS and IR are associated with glucose and lipid metabolism, respectively. Significant progress has been made in the identification of common and rare genetic variations associated with the MetS and IR in different populations, driven by the advent of GWAS and more recently, genome and exome sequencing approaches.

Despite many scientific efforts in identifying many genetic loci associated with the MetS and IR, their exact molecular pathogenesis remains unclear. Further studies are needed to identify functional links between the genetic variants and the phenotypes and subsequently to uncover the underlying molecular mechanisms of both metabolic disorders.

Clinical validation of the variants identified by several genetic analysis approaches is challenging for reasons resulting from implications by an individual's lifestyle and environmental factors as well as by genetic factors. In this aspect, studies including larger and more homogeneous populations are needed to identify genetic variants that underlie the association of the various traits of MetS and/or IR. However, results obtained from these studies should be replicated in different populations with a sufficient sample size to avoid false-positive associations and to reduce systematic biases and technical errors.

Approaches such as CRISPR, 3C, and eQTL are being used to identify structural and functional associations between genetic loci discovered by GWAS or exome sequencing and regional or distal genes. Among them, CRISPR as an *in vitro* screening platform may be used effectively to pinpoint causal genes at loci associated with MetS and IR in the near future. Currently, MetS and IR have been becoming a health and financial burden worldwide. The exact identification of validated variants that affect the MetS and IR might provide new preventive and treating strategies for the 2 metabolic disorders and related diseases.

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